

[2] Directory of Restriction Endonucleases

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Table I is intended to serve as a directory to the restriction endonucleases that have now been characterized. In forming the list, all endonucleases that cleave DNA at a specific sequence have been considered restriction enzymes, although in most cases there is no direct genetic evidence for the presence of a host-controlled restriction-modification system.

Certain strains have been omitted from this list to save space. Thus the many different *Staphylococcus aureus* isolates containing an isoschizomer of *Sau*3A¹ are not listed individually. Similarly the numerous strains of gliding bacteria (orders *Myxobacterales* and *Cytophagales*) that showed evidence of specific endonucleases during a large-scale screening² are still rather poorly characterized.

Within Table I the source of each microorganism is given either as an individual or a national culture collection. The enzymes are named in accordance with the proposal of Smith and Nathans.³ When two enzymes recognize the same sequence (i.e., are isoschizomers), the prototype (i.e., the first example isolated) is indicated in parentheses in column 3. The recognition sequences (column 4) are abbreviated so that only one strand, reading 5' → 3', is indicated and the point of cleavage, when known, is indicated by an arrow (↓). When two bases appear in parentheses, either one may appear at that position within the recognition sequence. Where known, the base modified by the corresponding methylase is indicated by an asterisk. \hat{A} is *N*⁶-methyladenosine; \hat{C} is 5-methylcytosine. The frequency of cleavage (columns 5–8) has been experimentally determined for bacteriophage lambda (λ) and adenovirus-2 (Ad2) DNAs, but represents the computer-derived values from the published sequences of SV40⁴ and φX174⁵ DNAs. When more than one reference appears (column 9), the first contains the purification procedure for the restriction enzyme, the second concerns its recognition sequence, the third contains the purification procedure for the methylase,

1. E. E. Stobbering, R. Schiphof, and J. S. Sussenbach, *J. Bacteriol.* **131**, 645 (1977).
2. H. Mayer and H. Reichenbach, *J. Bacteriol.* **136**, 708 (1978).
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4. V. B. Reddy, B. Thimmappaya, R. Dhar, K. N. Subramanian, B. S. Zain, J. Pan, P. K. Ghosh, M. L. Celma, and S. M. Weissman, *Science* **200**, 494 (1978).
5. F. Sanger, G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. Hutchison, III, P. M. Slocombe, and M. Smith, *Nature (London)* **265**, 687 (1977).

TABLE I
RESTRICTION ENDONUCLEASES

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites				References*
				λ	Ad2	SV40	ϕ X174	
<i>Achromobacter immobilis</i>	ATCC 15934	AimI	?	?	?	?	?	6
<i>Acinetobacter calcoaceticus</i>	R. J. Roberts	AccI	GT↓(C _T ^A)AC	7	8	1	3	7
<i>Agrobacterium tumefaciens</i>	R. J. Roberts	AccII (FnuDII)	CGCG	>50	>50	0	14	7
	ATCC 15955	AttAI	?	>30	>30	?	?	8
<i>Agrobacterium tumefaciens</i> B6806	E. Nester	AttBI (EcoRII)	CC↓(C _T ^A)GG	>35	>35	16	2	9
<i>Agrobacterium tumefaciens</i> ID 135	C. Kado	AttII (EcoRII)	CC↓(C _T ^A)GG	>35	>35	16	2	10
<i>Agrobacterium tumefaciens</i> C58	E. Nester	AttCI (BclI)	TGATCA	7	5	1	0	8
<i>Anabaena catanula</i>	CCAP 1403/1	AcaI	?	?	?	?	?	11
<i>Anabaena cylindrica</i>	A. de Waard	AcyI	GPu↓CGPyC	>14	>14	0	7	12
<i>Anabaena subcylindrica</i>	K. Murray	AsaI	G↓GNCC	>30	>30	11	2	11
<i>Anabaena variabilis</i>	K. Murray	AvaI	C↓PyCGPuG	8	?	0	1	13
<i>Anabaena variabilis</i> ^{uw}	K. Murray	AvaII	G↓G↓(C _T ^A)CC	>17	>30	6	1	13, 14 and 15
	K. Murray	AvaIII	ATGCAT	?	?	3	0	16, 17 and 18
<i>Arthrobacter luteus</i>	E. C. Rosenfold	AvrI (AvaI)	CPyCGPuG	8	?	0	1	19
	E. C. Rosenfold	AvrII	CCTAGG	1	2	2	0	19
<i>Arthrobacter pyridinolis</i>	ATCC 21606	AluI	AG↓CT	>50	>50	35	24	20
<i>Bacillus amyloliquefaciens</i> F	R. DiLauro	ApyI	CC↓(C _T ^A)GG	>35	>35	16	2	21
	ATCC 23350	BamFI (BamHI)	GGATCC	5	3	1	0	22
<i>Bacillus amyloliquefaciens</i> H	F. E. Young	BamHI	G↓GATCC	5	3	1	0	23, 24
<i>Bacillus amyloliquefaciens</i> K	T. Kaneko	BamKI (BamHI)	GGATCC	5	3	1	0	22
<i>Bacillus amyloliquefaciens</i> N	T. Ando	BamNI (BamHI)	GGATCC	5	3	1	0	25
<i>Bacillus brevis</i> S	T. Ando	BamN _x	?	?	?	?	?	25 and 26
	A. P. Zarubina	BbvSI	*T GC↓(C _T ^A)GC	Specific methylase				27
	ATCC 00099	BbvI	T GC↓(C _T ^A)GC	>30	>30	23	14	28

<i>Anabaena variabilis</i> ^{uv}	E. C. Rosenvold	AvrI (AvaI)	CPYCGPuG	8	?	0	1	19	
	E. C. Rosenvold	AvrII	CCTAGG	1	2	2	0	19	
<i>Arthrobacter luteus</i>	ATCC 21606	AluI	AG↓CT	>50	>50	35	24	20	
<i>Arthrobacter pyridinolis</i>	R. DiLauro	ApyI	CC↓GG	>35	>35	16	2	21	
<i>Bacillus amyloliquefaciens</i> F	ATCC 23350	BamFI (BamHI)	GGATCC	5	3	1	0	22	
<i>Bacillus amyloliquefaciens</i> H	F. E. Young	BamHI	G↓GATCC	5	3	1	0	23, 24	
<i>Bacillus amyloliquefaciens</i> K	T. Kaneko	BamKI (BamHI)	GGATCC	5	3	1	0	22	
<i>Bacillus amyloliquefaciens</i> N	T. Ando	BamNI (BamHI)	GGATCC	5	3	1	0	25	
	T. Ando	BamN ₈	?	?	?	?	?	25 and 26	
<i>Bacillus brevis</i> S	A. P. Zarubina	BbvSI	*T↓GC GC(↓)GC	Specific methylase					27
<i>Bacillus brevis</i>	ATCC 9999	BbvI	GC(↓)GC	>30	>30	23	14	28	
<i>Bacillus caldolyticus</i>	A. Atkinson	BclI	T↓GATCA	7	5	1	0	29	
<i>Bacillus cereus</i>	ATCC 14579	BceI	?	>10	?	?	?	22	
<i>Bacillus cereus</i>	IAM 1229	BceI229	?	>10	?	?	?	22	
<i>Bacillus cereus</i>	T. Ando	BceI70 (PstI)	CTGCAG	18	25	2	1	22	
<i>Bacillus cereus</i> Rf sm st	T. Ando	BceR (FnuDII)	CGCG	>50	>50	0	14	22	
<i>Bacillus globigii</i>	G. A. Wilson	BglI	GCCNNNN ↓ NGGC	22	12	1	0	30 and 31, 32	
	G. A. Wilson	BglI	A↓GATCT	>6	12	0	0	30 and 31, 33	
	B899	Bme899	?	>5	?	?	?	22	
<i>Bacillus megaterium</i> 899	T. Kaneko	Bme205	?	>10	?	?	?	22	
<i>Bacillus megaterium</i> B205-3	J. Upcroft	BmeI	?	>10	>20	4	?	34	
<i>Bacillus megaterium</i>	T. Ando	BpuI	?	6	>30	2	?	35	
<i>Bacillus pumilus</i> AHU1387	IAM 1286	BspI286	?	?	?	?	?	22	
<i>Bacillus sphaericus</i>	P. Venetianer	BspRI (HaeIII)	GGCC	>50	>50	19	11	36	
<i>Bacillus sphaericus</i> R	N. Welker	BsrI (BamHI)	GGATCC	5	3	1	0	37	
<i>Bacillus stearothermophilus</i>				?	?	?	?	38	
1503-4R	A. Atkinson	BstAI	?	?	?	?	?	39	
<i>Bacillus stearothermophilus</i> 240	N. Welker	BstEI	?	?	?	?	?	39	
<i>Bacillus stearothermophilus</i> ET	N. Welker	BstEII	?	11	8	0	0	39	
	N. Welker	BstEIII	?	>7	?	?	?	39	
<i>Bacillus subtilis</i> strain X5	T. Trautner	BsuRI (HaeIII)	GG↓CC	>50	>50	19	11	40, 41, 42	
<i>Bacillus subtilis</i> Marburg 168	T. Ando	BsuM	?	>10	?	?	?	22	
<i>Bacillus subtilis</i>	ATCC 6633	Bsu6663	?	>20	?	?	?	22	
<i>Bacillus subtilis</i>	IAM 1076	Bsu1076 (HaeIII)	GGCC	>50	>50	19	11	22	

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TABLE I—Continued

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites					References ^a
				λ	Ad2	SV40	ϕ X174		
<i>Bacillus subtilis</i>	IAM 1114	<i>Bsu</i> 1114 (<i>Hae</i> III)	GGCC	>50	>50	19	11		22
<i>Bacillus subtilis</i>	IAM 1247	<i>Bsu</i> 1247 (<i>Pst</i> I)	CTGCAG	18	25	2	1		22, 43
<i>Bacillus subtilis</i>	ATCC 14593	<i>Bsu</i> 1145	?	>20	?	?	?		22
<i>Bacillus subtilis</i>	IAM 1192	<i>Bsu</i> 1192	?	>10	?	?	?		22
<i>Bacillus subtilis</i>	IAM 1193	<i>Bsu</i> 1193	?	>30	?	?	?		22
<i>Bacillus subtilis</i>	IAM 1231	<i>Bsu</i> 1231	?	>20	?	?	?		22
<i>Bacillus subtilis</i>	IAM 1259	<i>Bsu</i> 1259	?	>8	?	?	?		22
<i>Bordetella bronchiseptica</i>	ATCC 19395	<i>Bbr</i> I (<i>Hind</i> III)	AAGCTT	6	11	6	0		44
<i>Brevibacterium albidum</i>	ATCC 15831	<i>Bal</i> I	TGG↓CCA	15	17	0	0		45
<i>Brevibacterium luteum</i>	ATCC 15830	<i>Blu</i> I (<i>Xho</i> I)	C↓TCGAG	1	5	0	1		46
<i>Caryophanon latum</i> L.	ATCC 15830	<i>Blu</i> II (<i>Hae</i> III)	GGCC	>50	>50	19	11		47
	H. Mayer	<i>Clal</i>	AT↓CGAT	12	?	0	0		48
<i>Chloroflexus aurantiacus</i>	A. Bingham	<i>Caul</i> (<i>Ava</i> II)	GGC↓CC	>30	>30	6	1		49
<i>Chromobacterium violaceum</i>	ATCC 12472	<i>Cau</i> II	?	>30	>30	0	?		49
<i>Corynebacterium humiferum</i>	ATCC 21108	<i>Cvi</i> I	?	?	?	?	?		6
	ATCC 21108	<i>Chu</i> I (<i>Hind</i> III)	AAGCTT	6	11	6	0		6
<i>Corynebacterium petrophilum</i>	ATCC 19080	<i>Chu</i> II (<i>Hind</i> II)	GTPyPuAC	34	>20	7	13		6
		<i>Cpe</i> I (<i>Bcl</i> I)	TGATCA	7	5	1	0		50
<i>Diplococcus pneumoniae</i>	S. Lacks	<i>Dpn</i> I	GA↓TC	?	?	?	0		51, 52 and 53
<i>Diplococcus pneumoniae</i>	S. Lacks	<i>Dpn</i> II (<i>Mbo</i> I)	GATC	>50	>50	7	0		51, 52
<i>Enterobacter cloacae</i>	H. Hartmann	<i>Ecl</i> II	?	15	?	?	?		54
	H. Hartmann	<i>Ecl</i> III (<i>Eco</i> RII)	CC↓GG	>35	>35	16	2		54
<i>Enterobacter cloacae</i>	DSM 30056	<i>Ecl</i> I	G↓GTNACC	12	?	0	0		55

Escherichia coli RY13

R. N. Yoshimori

*Eco*RI

G↓AAATTC

56, 57, 58

Escherichia coli R245

R. N. Yoshimori

*Eco*RII

↓CC↓GG

60, 61 and 62, 60

Escherichia coli B

W. Arber

*Eco*BTGA(N)₆TGCT

63, 64 and 65, 66

Escherichia coli K

M. Meselson

*Eco*KAAC(N)₆GTGC

67, 68, 69

Escherichia coli (PI)

K. Murray

*Eco*PI

AGACC

70, 71, 72 and 73, 74

<i>Diplococcus pneumoniae</i>	S. Lacks	DpnI	GA↓TC	?	?	?	0	51, 52 and 53
<i>Diplococcus pneumoniae</i>	S. Lacks	DpnII (MboI)	GATC	>50	>50	?	0	51, 52
<i>Enterobacter cloacae</i>	H. Hartmann	EclI	?	15	?	?	?	54
	H. Hartmann	EclII (EcoRII)	CC↓GG	>35	>35	16	2	54
<i>Enterobacter cloacae</i>	DSM 30056	EcaI	G↓GTNACC	12	?	0	0	55

<i>Escherichia coli</i> RY13	R. N. Yoshimori	EcoRI	G↓AATTC	5	5	1	0	56, 57, 56, 58
	R. N. Yoshimori	EcoRI'	PuPuA↓TPyPy	>10	>10	24	16	59
<i>Escherichia coli</i> R245	R. N. Yoshimori	EcoRII	↓CC↓GG	>35	>35	16	2	60, 61 and 62, 60
<i>Escherichia coli</i> B	W. Arber	EcoB	TGA(N)↓TGCT	?	?	?	?	63, 64 and 65, 66
<i>Escherichia coli</i> K	M. Meselson	EcoK	AAC(N)↓GTGC	?	?	?	?	67, 68, 69
<i>Escherichia coli</i> (PI)	K. Murray	EcoPI	AGACC	?	?	?	?	70, 71, 72 and 73, 74
<i>Escherichia coli</i> P15	W. Arber	EcoP15	?	?	?	?	?	75
<i>Fusobacterium nucleatum</i> A	M. Smith	FnuAI (HinfI)	G↓ANTC	>50	>50	10	21	76
	M. Smith	FnuAII (MboI)	GATC	>50	>50	7	0	44
<i>Fusobacterium nucleatum</i> C	M. Smith	FnuCI (MboI)	↓GATC	>50	>50	7	0	76
<i>Fusobacterium nucleatum</i> D	M. Smith	FnuDI (HaeIII)	GG↓CC	>50	>50	19	11	76
	M. Smith	FnuDII	CG↓CG	>50	>50	0	14	76
	M. Smith	FnuDIII (HhaI)	GCG↓C	>50	>50	2	18	76
<i>Fusobacterium nucleatum</i> E	M. Smith	FnuEI (SmaI)	↓GATC	>50	>50	7	0	76
<i>Fusobacterium nucleatum</i> 48	M. Smith	Fnu48 I	?	>50	?	?	?	76
<i>Haemophilus aegyptius</i>	ATCC 11116	HaeI	↓GG↓CC↓	?	?	11	6	77
	ATCC 11116	HaeII	PuGCGC↓Py	>30	>30	1	8	78, 79
<i>Haemophilus aphrophilus</i>	ATCC 11116	HaeIII	GG↓CC	>50	>50	19	11	80, 41, 81
	ATCC 19415	HapI	?	>30	?	?	?	44
	ATCC 19415	HapII (HpaII)	C↓CGG	>50	>50	1	5	82, 83
<i>Haemophilus gallinarum</i>	ATCC 14385	HhaI*	GACGC	>50	>50	0	14	82, 84 and 85
<i>Haemophilus haemoglobinophilus</i>	ATCC 19416	HhgI (HaeIII)	GGCC	>50	>50	19	11	44
<i>Haemophilus haemolyticus</i>	ATCC 10014	HgaI	G↓CG↓C	>50	>50	2	18	86, 86, 87
	ATCC 10014	HhaII (HinfI)	GATC	>50	>50	10	21	88
<i>Haemophilus influenzae</i> 1056	J. Stuy	Hin 1056I (FnuDII)	CGCG	>50	>50	0	14	89
	J. Stuy	Hin 1056II	?	>30	>30	0	4	89

(Continued)

TABLE 1—Continued

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites				References ^c
				λ	Ad2	SV40	ϕ X174	
<i>Haemophilus influenzae</i> serotype b, 1076	J. Stuy	HinbIII (<i>Hind</i> III)	AAGCTT	6	11	6	0	89
<i>Haemophilus influenzae</i> R _b	C. A. Hutchison	HinbIII (<i>Hind</i> III)	AAGCTT	6	11	6	0	90 and 42
<i>Haemophilus influenzae</i> serotype c, 1160	J. Stuy	HincII (<i>Hind</i> II)	GTPyPuAC	34	>20	7	13	89
<i>Haemophilus influenzae</i> serotype c, 1161	J. Stuy	HincII (<i>Hind</i> II)	GTPyPuAC	34	>20	7	13	89
<i>Haemophilus influenzae</i> R _c	A. Landy, G. Leidy	HincII (<i>Hind</i> II)	GTPyPuAC	34	>20	7	13	91
<i>Haemophilus influenzae</i> R _d (exo mutant)	S. H. Goodgal	HindI	CAC	Specific methylase				92, 93
	S. H. Goodgal	HindII	GTPy ↓ PuAC	34	>20	7	13	94, 95, 92, 93
	S. H. Goodgal	HindIII	*A ↓ AGCTT	6	11	6	0	96, 96, 92, 93
	S. H. Goodgal	HindIV	GAC	Specific methylase				92, 93
<i>Haemophilus influenzae</i> R _d 123	V. Tanyashin	HindGLU	?	?	?	?	?	97
<i>Haemophilus influenzae</i> R _t	C. A. Hutchison	HinfI	G ↓ ANTC	>50	>50	10	21	90, 98 and 99
<i>Haemophilus influenzae</i> H-1	C. A. Hutchison	HinfII (<i>Hind</i> III)	AAGCTT	6	11	6	0	87
<i>Haemophilus parahaemolyticus</i>	M. Takanami	HinHI (<i>Hae</i> II)	PuGCGCPy	>30	>30	1	8	82
<i>Haemophilus parainfluenzae</i>	C. A. Hutchison	HphI ^b	GGTGA	>50	>50	4	9	90, 100
	J. Setlow	HpaI	GTT ↓ AAC	11	6	4	3	101, 102
<i>Haemophilus suis</i>	J. Setlow	HpaII	C ↓ CGG	>50	>50	1	5	101, 102, 81
<i>Herpetosiphon giganteus</i> HP1023	ATCC 19417	HsaI (<i>Hind</i> III)	A ↓ AGCTT	6	11	6	0	44
<i>Klebsiella pneumoniae</i> OK8	J. H. Parish	HgiAI	G()GCG() ↓ C	20	?	0	3	103
<i>Microcoleus species</i>	J. Davies	KpnI	GGTAC ↓ C	2	8	1	0	104, 105
	D. Comb	MstI	TGCGCA	>10	>15	0	1	106, 106a

<i>Moraxella bovis</i>	ATCC 10900	MboI	↓ GATC	>50	>50	7	0	107
	ATCC 10900	MboII ^c	GAAGA	>50	>50	15	11	107, 108 and 109
<i>Moraxella glucidi</i> LG1	J. Davies	MglI	?	?	?	?	?	104
<i>Moraxella glucidi</i> LG2	J. Davies	MglII	?	?	?	?	?	104
<i>Moraxella nonliquefaciens</i>	ATCC 19975	MnoI (<i>Hpa</i> II)	C ↓ CGG	>50	>50	1	5	44, 110
	ATCC 19975	MnoII	?	>10	>6	2	?	44
<i>Moraxella nonliquefaciens</i>	ATCC 17953	MnlI ^d	CCTC	>100	>100	52	35	111
<i>Moraxella nonliquefaciens</i>	ATCC 17954	MnnI (<i>Hind</i> II)	GTPyPuAC	34	>20	7	13	112
<i>Moraxella nonliquefaciens</i>	ATCC 17954	MnnII (<i>Hae</i> III)	GGCC	>50	>50	19	11	112

Haemophilus suis	ATCC 19417	HsuI (HindIII)	A ↓ AGCTT	0	?	0	3	103
Herpetosiphon giganteus	J. H. Parish	HgiAI	G ^T (GCT) ↓ C _A	20	?	0	3	103
HP1023	J. Davies	KpnI	GGTAC ↓ C	2	8	1	0	104, 105
Klebsiella pneumoniae OK8	D. Comb	MstI	TGCGCA	>10	>15	0	1	106, 106a
Microcoleus species								
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Moraxella bovis	ATCC 10900	MboI	↓ GATC	>50	>50	7	0	107
	ATCC 10900	MboII ^c	GAAGA	>50	>50	15	11	107, 108 and 109
	J. Davies	MgII	?	?	?	?	?	104
Moraxella glucidi LG1	J. Davies	MgIII	?	?	?	?	?	104
Moraxella glucidi LG2	ATCC 19975	MnoI (HpaII)	C ↓ CGG	>50	>50	1	5	44, 110
Moraxella nonliquefaciens	ATCC 19975	MnoII	?	>10	>6	2	?	44
	ATCC 17953	MniI ^d	CGTC	>100	>100	52	35	111
Moraxella nonliquefaciens	ATCC 17953	MnnI (HindIII)	GTPyPuAC	34	>20	7	13	112
Moraxella nonliquefaciens	ATCC 17954	MnnII (HaeIII)	GGCC	>50	>50	19	11	112
	ATCC 17954	MnnIII	?	>50	>50	?	?	112
	ATCC 17954	MnnIV (HhaI)	GCGC	>50	>50	2	18	112
	ATCC 17954	MosI (MboI)	GATC	>50	>50	7	0	107
	ATCC 19976	MspI (HpaII)	CCGG	>50	>50	1	5	113
Moraxella osloensis	R. J. Roberts	MviI	?	1	?	?	?	114
Moraxella species	H. Reichenbach	MviII	?	?	?	?	?	114
Myxococcus virescens	H. Reichenbach	MviII	?	?	?	?	?	114
	G. Wilson	NgoI (HaeII)	PuGCGPy	>30	>30	1	8	115
Neisseria gonorrhoea	CDC 66	NgoII (HaeIII)	GGCC	>50	>50	19	11	116
Neisseria gonorrhoea	R. Shekman	OxaI (AlaI)	AGCT	>50	>50	35	24	117
Oerskovia xanthineolytica	R. Shekman	OxaII	?	?	?	?	?	117
	ATCC 13315	PvuI	CGATCG	4	7	0	0	28
Proteus vulgaris	ATCC 13315	PvuII	CAG ↓ CTG	15	22	3	0	28
	ATCC 9886	Pall (HaeIII)	GGCC	>50	>50	19	11	34
Providencia alcalifaciens	J. Davies	PstI	CTGCA ↓ G	18	25	2	1	104, 118
Providencia stuartii 164	M. VanMontagu	Pfal	?	>30	>30	?	?	47, 89
Pseudomonas facilis	R. Lascelles	RspI	?	3	12	0	?	119
Rhodopseudomonas sphaeroides	S. Kaplan	RshI (PvuI)	CGATCG	4	7	0	0	120
Rhodopseudomonas sphaeroides	C. Mulder	SmaI	CCC ↓ GGG	3	12	0	0	121, 122
Serratia marcescens S ₆	B. Torheim	SspI	?	?	?	?	?	123
Serratia species SAI	E. E. Stobberingh	Sau3A (MboI)	GATC	>50	>50	7	0	124
Staphylococcus aureus 3A	E. E. Stobberingh	Sau96I (AvaI)	G ↓ GNCC	>30	>30	11	2	125
Staphylococcus aureus PS96								

(Continued)

TABLE 1—Continued

Microorganism	Source	Enzyme	Sequence	Number of cleavage sites					References ^a
				λ	Ad2	SV40	ϕ X174		
<i>Streptococcus faecalis</i> subsp. <i>zymogenes</i>	R. Wu	<i>Sfa</i> I (<i>Hae</i> III)	GG \downarrow CC	>50	>50	19	11	126	
<i>Streptococcus faecalis</i> ND547	D. Clewell	<i>Sfa</i> NI	GATGC	>50	>30	6	12	8	
<i>Streptomyces achromogenes</i>	ATCC 12767	<i>Sac</i> I	GAGCT \downarrow C	2	7	0	0	127	
	ATCC 12767	<i>Sac</i> II	CCGC \downarrow GG	3	>25	0	1	127	
	ATCC 12767	<i>Sac</i> III	?	>30	>30	?	?	127	
	CM1 52766	<i>Sa</i> IP1 (<i>Pst</i> I)	CTGCAG	18	25	2	1	128	
<i>Streptomyces albus</i>	KCC S0166	<i>Spa</i> I (<i>Xho</i> I)	CTCGAG	1	5	0	1	129	
<i>Streptomyces albus</i> subsp. <i>pathociclicus</i>	J. M. Ghuyssen	<i>Sa</i> II	G \downarrow TCGAC	2	3	0	0	130	
<i>Streptomyces bobilliae</i>	J. M. Ghuyssen	<i>Sa</i> II	?	>30	?	?	?	130	
	ATCC 3310	<i>Sbo</i> I	?	?	?	?	?	131	
	ATCC 3535	<i>Sbr</i> I	?	?	?	?	?	131	
	KCC S0316	<i>Sca</i> I (<i>Xho</i> I)	CTCGAG	1	5	0	1	131	
<i>Streptomyces cupidosporus</i>	H. Takahashi	<i>Sex</i> I (<i>Xho</i> I)	CTCGAG	1	6	0	1	129	
<i>Streptomyces exfoliatus</i>	H. Takahashi	<i>Sgo</i> I (<i>Xho</i> I)	CTCGAG	1	6	0	1	129	
<i>Streptomyces goshikiensis</i>	ATCC 23345	<i>Sgr</i> I	?	0	7	0	?	127	
<i>Streptomyces griseus</i>	?	<i>Shy</i> I	?	2	?	?	?	132	
<i>Streptomyces hygroscopicus</i>	ATCC 8644	<i>Sla</i> I (<i>Xho</i> I)	C \downarrow TCGAG	1	6	0	1	131	
<i>Streptomyces lavendulae</i>	H. Takahashi	<i>Slu</i> I (<i>Xho</i> I)	CTCGAG	1	6	0	1	129	
<i>Streptomyces luteoreticuli</i>	S. Goff,	<i>Ssf</i> I (<i>Sac</i> I)	GAGCT \downarrow C	2	7	0	0	133, 134	
<i>Streptomyces stanford</i>	A. Rambach	<i>Ssr</i> II (<i>Sac</i> II)	CCGC \downarrow GG	3	>25	0	1	133	
	S. Goff,	<i>Ssr</i> III (<i>Sac</i> III)	?	>30	>30	?	?	133	
	A. Rambach								

<i>Thermoplasma acidophilum</i>	D. Searcy	<i>Tha</i> I (<i>Fnu</i> DII)	CG \downarrow CG	>50	>50	0	14	135	
<i>Thermoplasma glauca</i>	ATCC 15345	<i>Tg</i> II (<i>Sac</i> II)	CCGCGG	3	>25	0	1	28	
<i>Thermus aquaticus</i> YTI	J. I. Harris	<i>Taq</i> I	T \downarrow CGA	>50	>50	1	10	136	
<i>Xanthomonas amaranthicola</i>	J. I. Harris	<i>Taq</i> II	?	>30	>30	4	6	44	
<i>Xanthomonas badrii</i>	ATCC 11645	<i>Xan</i> I (<i>Sa</i> I)	GTGAC	2	3	0	0	130	
<i>Xanthomonas holcicola</i>	ATCC 11672	<i>Xba</i> I	T \downarrow CTAGA	1	4	0	0	137	
	ATCC 13461	<i>Xho</i> I	C \downarrow TCGAG	1	6	0	1	46	
	ATCC 13461	<i>Xho</i> II	Pu \downarrow GATCPy	>20	>20	?	?	90	

<i>Streptomyces stanford</i>	S. Goff,	SstI (SacI)	GAGCT↓C	2	7	0	0	133, 134
	A. Rambach							
	S. Goff,	SstII (SacII)	CCGC↓GG	3	>25	0	1	133
	A. Rambach							
	S. Goff,	SstIII (SacIII)	?	>30	>30	?	?	133
	A. Rambach							

<i>Thermoplasma acidophilum</i>	D. Searey	<i>ThaI</i> (FnuDII)	CG↓CG	>50	>50	0	14	135
<i>Thermopolyspora glauca</i>	ATCC 15345	<i>TgI</i> (SacII)	CCGCGG	3	>25	0	1	28
<i>Thermus aquaticus</i> YTI	J. I. Harris	<i>TaqI</i>	T↓CGA	>50	>50	1	10	136
	J. I. Harris	<i>TaqII</i>	?	>30	>30	4	6	44
<i>Xanthomonas anaranthicola</i>	ATCC 11645	<i>XamI</i> (Sall)	GTCGAC	2	3	0	0	130
<i>Xanthomonas badrii</i>	ATCC 11672	<i>XbaI</i>	T↓CTAGA	1	4	0	0	137
<i>Xanthomonas holcicola</i>	ATCC 13461	<i>XhoI</i>	C↓TCGAG	1	6	0	1	46
	ATCC 13461	<i>XhoII</i>	Pu↓GATCpy	>20	>20	3	0	89, 28
<i>Xanthomonas malvacearum</i>	ATCC 9924	<i>XmaI</i>	C↓CCGGG	3	12	0	0	122
	ATCC 9924	<i>XmaII</i> (PstI)	CTGCAG	18	25	2	1	122
<i>Xanthomonas nigromaculans</i>	ATCC 23390	<i>XniI</i> (PvuI)	CGATCG	4	7	0	0	112
<i>Xanthomonas oryzae</i>	M. Ehrlich	<i>XorI</i> (PstI)	CTGCAG	18	25	2	1	138
	M. Ehrlich	<i>XorII</i> (PvuI)	CGATCG	4	7	0	0	138
<i>Xanthomonas papavericola</i>	ATCC 14180	<i>XpaI</i> (XhoI)	C↓TCGAG	1	6	0	1	138

- ^a *HgaI* cleaves as indicated: 5' GACGNNNNN ↓ 3'
- ^b *HphI* cleaves as indicated: 5' GGTGNNNNNNNN ↓ 3'
- ^c *MboII* cleaves as indicated: 5' GAAGNNNNNNNN ↓ 3'
- ^d *MniI* cleaves 5 to 10 bases from the recognition sequence.
- ^e Key to references:

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TABLE II
LIST OF ENZYMES WITH KNOWN RECOGNITION SEQUENCES

Terminal extension	Restriction enzyme	Recognition sequence
Blunt ends	<i>DpnI</i>	GA [*] ↓TC
	<i>EcoRI</i> ¹	PuPuA↓TPyPy
	<i>SmaI</i>	CCC↓GGG
	<i>AluI</i>	AG↓CT
	<i>PvuII</i>	CAG↓CTG
	<i>FnuDII</i>	CG↓CG
	<i>HaeI</i>	$\begin{smallmatrix} \text{A} & & \text{A} \\ \diagdown & & / \\ \text{GG} & \downarrow & \text{CC} \\ \diagup & & \diagdown \\ \text{T} & & \text{T} \end{smallmatrix}$
	<i>HpaI</i>	GTT↓AAC
5'↓GATC	<i>MboI</i>	↓GATC
	<i>BglII</i>	A↓GATCT
	<i>BamHI</i>	G↓GATCC
	<i>BclI</i>	T↓GATCA
	<i>XhoII</i>	Pu↓GATCPy
5'↓CG	<i>HpaII</i>	C↓CGG
	<i>TaqI</i>	T↓CGA
	<i>ClaI</i>	AT↓CGAT
	<i>AcyI</i>	GPu↓CGPyC
5'↓TCGA	<i>XhoI</i>	C↓TCGAG
	<i>SalI</i>	G↓TCGAC
5'↓AATT	<i>EcoRI</i>	G↓AATTC
5'↓AGCT	<i>HindIII</i>	A↓AGCTT
5'↓CCGG	<i>XmaI</i>	C↓CCGGG
5'↓CTAG	<i>XbaI</i>	T↓CTAGA
3' TGCA↓	<i>PstI</i>	CTGCA↓G
3' GTAC↓	<i>KpnI</i>	GGTAC↓C
3' GC↓	<i>SacII</i>	CCGC↓GG
3' GCGC↓	<i>HaeII</i>	PuGCGC↓Py
3' CG↓	<i>HhaI</i>	GCG↓C
3' AGCT↓	<i>SacI</i>	GAGCT↓C
5'↓CC $\begin{smallmatrix} \text{A} \\ \diagdown \\ \text{T} \end{smallmatrix}$ GG	<i>EcoRII</i>	↓CC $\begin{smallmatrix} \text{A} \\ \diagdown \\ \text{T} \end{smallmatrix}$ GG
5'↓GTNAC	<i>EcaI</i>	G↓GTNACC
5'↓NNNNN	<i>HgaI</i>	5'↓NNNNNNNNNNGCGTC 3' 3' ↑NNNNNCGCAG 5'
5'↓PyCGPu	<i>AvaI</i>	C↓PyCGPuG
5'↓ANT	<i>HinII</i>	G↓ANTC
5'↓GNC	<i>AsuI</i>	G↓GNCC
5'↓G $\begin{smallmatrix} \text{A} \\ \diagdown \\ \text{T} \end{smallmatrix}$ C	<i>AvaII</i>	G↓G $\begin{smallmatrix} \text{A} \\ \diagdown \\ \text{T} \end{smallmatrix}$ CC
5'↓ $\begin{smallmatrix} \text{A} & \text{G} \\ \diagdown & \diagup \\ \text{C} & \text{T} \end{smallmatrix}$	<i>AccI</i>	GT↓ $\begin{smallmatrix} \text{A} & \text{G} \\ \diagdown & \diagup \\ \text{C} & \text{T} \end{smallmatrix}$ AC
3' N↓	<i>HphI</i>	5' GGTGANNNNNNNN ↓ 3' 3' CCACTNNNNNNNN ↑ 5'
	<i>MboII</i>	5' GAAGANNNNNNNN ↓ 3' 3' CTTCTNNNNNNNN ↑ 5'

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[3] Addition of Duplex

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¹ This work was sup-
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² I. R. Lehman, *Science*

³ J. E. Mertz and R. V.

⁴ P. E. Lobban and A.

⁵ D. A. Jackson, R. H.

⁶ P. C. Wensink, D. J.

and the fourth describes its recognition sequence. In some cases two references appear in one of these categories when two independent groups have reached similar conclusions.

Table II contains a listing of enzymes for which the recognition sequence is known and which might be useful for preparing recombinant DNAs. They are grouped according to the nature of the fragment ends produced. Thus, fragments generated by all enzymes within any group can be joined to one another.

[3] Addition of Homopolymers to the 3'-Ends of Duplex DNA with Terminal Transferase¹

By TIMOTHY NELSON and DOUGLAS BRUTLAG

The linkage of two DNAs *in vitro* to form recombinant molecules first became possible with the discovery of DNA ligases.² These enzymes, which seal nicks in DNA, can covalently join two DNAs that have complementary sticky ends such as the short, staggered ends generated by many restriction endonucleases.³ Lobban and Kaiser⁴ and Jackson *et al.*⁵ showed that complementary ends could be added to DNA molecules *in vitro* with terminal transferase, thus allowing any two DNAs to be linked. These workers added complementary single-stranded homopolymers to two DNA molecules, annealed the homopolymer regions, and covalently closed the resulting hybrid *in vitro* with DNA polymerase I and DNA ligase from *Escherichia coli*. The DNA polymerase was necessary to trim any excess unpaired nucleotides at the 3'-ends or to fill in gaps generated by unequal lengths of the complementary homopolymer regions. Wensink *et al.*⁶ simplified this procedure by showing that the annealed recombinant molecules were infectious and that they would be covalently closed *in vivo* during transfection.

Lobban and Kaiser⁴ originally found that completely duplex molecules were inefficient primers for the terminal transferase reaction and that pretreatment of the DNA with lambda exonuclease to expose single-stranded

¹ This work was supported by a Basil O'Connor starter grant from the National Foundation-March of Dimes.

² I. R. Lehman, *Science* **186**, 790 (1974).

³ J. E. Mertz and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3370 (1972).

⁴ P. E. Lobban and A. D. Kaiser, *J. Mol. Biol.* **78**, 453 (1973).

⁵ D. A. Jackson, R. H. Symons, and P. Berg, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2904 (1972).

⁶ P. C. Wensink, D. J. Finegan, J. E. Donelson, and D. S. Hogness, *Cell* **3**, 315 (1974).

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